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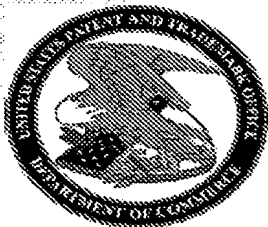
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INVENTOR(S)					
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<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max)					
ELECTROWETTING SAMPLE PRESENTATION DEVICE FOR MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY					
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Respectfully submitted,

SIGNATURE

Mara Featherstone

Date

8/22/03

REGISTRATION NO.

50,390

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Docket Number:

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Invention: ELECTROWETTING SAMPLE PRESENTATION DEVICE FOR MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY			
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PROVISIONAL PATENT APPLICATION

**ELECTROWETTING SAMPLE PRESENTATION DEVICE
FOR MATRIX-ASSISTED LASER DESORPTION/IONIZATION
MASS SPECTROMETRY**

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BACKGROUND OF THE INVENTION

Abstract of the Invention

[1] The present invention relates to electro-wettable sample presentation devices useful in performing analytical measurements configured to enable liquid drop manipulation so as to enhance detecting analytes contained within a liquid sample drop. The sample presentation device of the present invention is comprised of a physical or virtual microwell which can receive a liquid sample drop; one or more intermediary electro-wettable sites at least one of which is contiguous to the microwell; and a terminal electro-wettable site which confines the deposition of analytes and matrix to within a predetermined area. Preferably, the surface of the microwell is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes, or hydrophobic and adsorptive properties with respect to analytes. Each of the electro-wettable sites modifies the surface of the sample presentation device between hydrophobic and hydrophilic states in response to an electrical potential applied between a liquid sample drop and the electro-wettable site so as to direct the positioning of the liquid sample drop. Furthermore, with respect to the path which originates at the microwell, the surface area of each succeeding intermediary electro-wettable site is equal to or less than that of the preceding electro-wettable site.

Field of the Invention

[2] The present invention relates to the field of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). More particularly, the present invention relates to sample preparation devices and methods for use in matrix-assisted laser desorption/ionization mass spectrometry with improved analytical detection capabilities.

Background of the Invention

[3] Matrix-assisted laser desorption/ionization mass spectrometry is an important analytical tool in proteomics efforts, it that they are dependent upon the ability to rapidly analyze minute quantities of peptide and protein mixtures. Large scale proteomics applications require high sensitivity detection and high sample throughput at moderate cost. These demands

underscore the importance of sample preparation and automated data acquisition. In MALDI-MS, analytes are mixed with a matrix solution in an appropriate solvent and deposited on a sample support (also referred to as plate or target) for subsequent drying and crystallization. During the course of drying, crystal growth is induced and analyte molecules become co-crystallized with the matrix. The MALDI-MS sample support is then inserted into a mass spectrometer and a relatively small diameter (e.g. 100 μm) laser beam is directed onto the sample. Photon bombardment causes the analyte and matrix molecules to be desorbed and ionized without substantially fragmentation. The desorbed ions are then mass analyzed in the mass spectrometer. The matrix is an energy absorbing substance which absorbs energy from the laser beam thereby enabling desorption of analytes from the sample support.

[4] Present day MALDI-MS sample supports suffer from a severe limitation with respect to the liquid sample volume which may be applied to the support. Volumes in the range 0.5 to 3.0 μL are routinely utilized and afford dried-droplets containing analytes and matrix having diameters of from 1 to 2 mm. As a result, only a minute portion of the dried-droplet is irradiated by the laser during single-site data acquisition. Unfortunately, even small volumes of less than 3.0 μL are known to result in sample heterogeneity, which gives rise to significant variations in peak intensity, resolution and mass accuracy when focusing the laser on different regions of the dried-droplet (Strupat, K.; Karas, M.; Hillenkamp, F. *Int'l. J. Mass Spectrom. Ion Processes*, 1991, 111, 89-102; Cohen, S. L. and Chait, B. T. *Anal. Chem.*, 1996, 68, 31-37; and Amado, F. M. L.; Domingues, P.; Santana-Marques, M. G.; Ferrer-Correia, A. J.; Tomer, K. B. *Rapid Commun. Mass Spectrom.*, 1997, 11, 1347-1352, all of which are incorporated herein by reference). Sample heterogeneity results from differential precipitation of analytes which occurs during drying when volatile organic solvent present in the liquid sample drop rapidly evaporates rendering specific analytes insoluble. These phenomena render necessary the critical inspection of the mass spectral data as well as the accumulation of a large number of single-site spectra per sample. Consequently, only a few hundred samples can be analyzed per instrument per day, and automated data acquisition is often precluded.

[5] The problem of sample heterogeneity can be overcome or significantly reduced if the spot diameter falls below 250 μm . In this instance, a large portion of the sample can be irradiated simultaneously, improving sensitivity and reproducibility (Little, D. P.; Cornish, T. J.; O'Donnell, M. J.; Braun, A.; Cotter, R. J.; Koster, H. *Proc. Natl. Acad. Sci. U.S.A.*, 1997, 69,

4540-4546; and Gobom, J.; Nordhoff, E.; Mirgorodskaya, E.; Ekman, R.; Roepstorff, P. *J. Mass Spectrom.*, 1999, 34, 105-116, incorporated herein by reference).

[6] Peptide and protein samples purified by conventional liquid chromatographic and electrophoretic methods are routinely recovered in volumes of from 5 to 10 μ L, necessitating their concentration prior to MALDI-MS. Furthermore, many samples contain detergents and salts that interfere with mass spectral analyses and must be removed. To address these concerns, researchers have prepared micro-columns for sample concentration and desalting by packing small pipette tips with reverse phase chromatographic media (Rusconi, F.; Schmitter, J.-M.; Rossier, J.; le Maire, M. *Anal. Chem.*, 1998, 70, 3046-3052, incorporated herein by reference). Analytes are routinely recovered from micro-columns in volumes of from 5 to 10 μ L, and a portion of the eluant deposited onto the mass spectrometer sample support. The popularity of micro-column approaches prompted improvements in their design which modestly reduced the volume of eluant required to recover analytes to approximately 5 μ L (United States Patents Nos. 6,048,457 and 6,200,474, incorporated herein by reference). Micro-columns for MALDI-MS sample preparation are commercially available as ZipTips[®] from Millipore Corporation. However, the use of either ZipTips[®] or home-made micro-columns is time consuming, adds considerable cost, has proven difficult to automate and often affords only moderate (40 to 60%) recoveries of sample material. Nevertheless, micro-column approaches are routinely utilized to reduce sample volumes prior to MALDI-MS.

[7] Some of the limitations associated with the dried-droplet approach have been addressed, at least in part, by the MALDI-MS sample supports described in United States Patent No. 6,287,872, incorporated herein by reference. These sample supports are coated with a thin layer of nonwettable hydrophobic material that carries an array of 200 μ m diameter wettable hydrophilic spots. The invention exploits the "anchoring" attributes of the hydrophilic spots to direct the deposition of analytes to within an area with a diameter of less than 250 μ m. Aspects of the aforementioned sample supports are further described in scientific publications (Schüerenberg, M.; Lubbert, C.; Eickhoff, H.; Kalkum, M.; Lehrach, H.; Nordhoff, E. *Anal. Chem.* 2000, 72, 3436-3442; and Nordhoff, E.; Schüerenberg, M.; Thiele, G.; Lübbert, C.; Kleoppel, K.-D.; Theiss, D.; Lehrach, H. and Gobom, J. *Intl. Journal Mass Spectrometry* 2003, 226, 163-180, both of which are incorporated herein by reference). These articles conclude that confining the deposition of analytes to a small spot diameter not only reduces problems

associated with sample heterogeneity, but also results in a significant increase in sensitivity of detection.

[8] Sample supports which exploit small hydrophilic anchors are commercially available as the AnchorChip™ from Bruker Daltronics GmbH. Unfortunately; the manufacturer recommends that relatively large anchors of either 400 μm or 600 μm diameter be utilized for proteomics-related applications (AnchorChip™ Technology, Revision 1.6, Bruker Daltronics GmbH, Nov. 2000, incorporated herein by reference). Consequently, many of the difficulties outlined above regarding sample heterogeneity remain unaddressed. A further limitation associated with the use of the AnchorChip™ is the requirement that the volume of liquid sample applied to each anchor be limited to from 0.5 to 3.0 μL (No. 1 of Eleven General Rules for Sample Preparation on AnchorChip™ Targets). Furthermore, the examples provided by the manufacturer recommend limiting the liquid sample drop volume to either 0.5 or 1.0 μL (which is mixed with 2.0 μL of matrix solution prior to deposition on the AnchorChip™). Additionally, it is required that the sample first be purified and concentrated on a ZipTip® prior to application. Therefore, although still representing an improvement of sorts, the AnchorChip™ suffers many of the same limitations associated with other present day MALDI-MS sample supports.

[9] The general concept of exploiting wettable hydrophilic regions in conjunction with non-wettable hydrophobic films to confine samples to predetermined locations is described in United States Patents Nos. 5,041,266; 5,831,184; 5,958,345; and 6,555,813, all of which are incorporated herein by reference.

[10] Collectively, present day MALDI-MS sample supports suffer from a severe sample volume limitation, in that they are incompatible with sample volumes in excess of 3 μL . This volume is significantly smaller than the volume in which samples are routinely recovered from chromatographic or electrophoretic purifications, necessitating their further concentration prior to MALDI-MS. Furthermore, even volumes as small as 3 μL can prove problematic owing to sample heterogeneity when the dried-droplet approach is utilized. Although the AnchorChip™ addresses some of the problems associated with sample heterogeneity by reducing the sample spot size, it suffers from the same liquid sample volume constraints as other present day sample supports.

[11] In recent years, the principles of continuous electrowetting (CEW) and electrowetting-on-dielectric (EWOD) have attracted interest for microscale liquid handling (Washizu, M. *IEEE Transactions on Industry Applications*, 1998, 34(4), 732-737; Pollack, M. G.; Fair, R. B. and Shenderov, A. D. *Applied Physics Letters*, 2000, 77(11), 1725-1726; Lee, J.; Moon, H.; Fowler, J.; Schoelhammer, T. and Kim, C.-J. *Sensors and Actuators A*, 2002, 95, 259-268; Moon, H.; Cho, S. K.; Garrell, R. L. and Kim, C.-J. *J. Applied Physics*, 2002, 92(7), 4080-4087, all of which are incorporated herein by reference). CEW and EWOD are principles that can control wettability of solid surfaces with respect to conducting liquid drops by exploiting an electric potential. While CEW controls wettability of an electrolyte droplet on a metal electrode by varying electric energy across the electrical double layer, EWOD applies to virtually any aqueous droplet by varying electric energy across the thin dielectric film between the liquid and an electrode. By electrically changing the wettability of each of the electrodes patterned on a substrate, liquid drops can be shaped and driven along a series of electrodes, making microscale liquid handling extremely simple both with respect to device fabrication and operation. Several operations involving creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation have very recently been demonstrated (Cho, S. K.; Moon, H. and Kim, C.-J. *J. Microelectromechanical Systems*, 2003, 12(1), 70-80, incorporated herein by reference).

[12] Electrowetting-based electrostatic actuators for microfluidics are disclosed in United States Patent Application No. 2002/0043463, dated April 18, 2002, and United States Patent No. 6,565,727, dated May 20, 2003, both of which are incorporated herein by reference. Electrowetting-driven micropumping is disclosed in International Publication No. WO 02/07503, dated January 31, 2002, which is incorporated herein by reference. Never-the-less, the principle of electrowetting has not heretofore been exploited to address the particular sample preparation challenges associated with MALDI-MS.

[13] Therefore, a need exists for a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry that: (1) Is compatible with the sample volumes routinely recovered from liquid chromatographic and electrophoretic separations; (2) Directs the deposition of analytes to within a confined area so as to address those issues which result from sample heterogeneity; (3) Affords an increase in sensitivity of detection; and (4) Facilitates automated acquisition of data. The availability of such a sample presentation device would

enable automated sample processing on the life science industry's standard multiwell plate processors and liquid handling robots as well as enable the direct collection and subsequent analysis of chromatographic eluants by MALDI-MS. Collectively, these capabilities would significantly enhance the throughput of proteomics efforts.

SUMMARY OF THE INVENTION

[14] It is an object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry which is optimal with respect to receipt and subsequent positioning of a liquid sample drop.

[15] It is a further object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry which is optimal with respect to confining the co-deposition of analytes and matrix.

[16] It is another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry so as to facilitate the homogeneous co-deposition of analytes and matrix.

[17] It is another object of the present invention to provide a sample presentation device for matrix assisted laser desorption/ionization mass spectrometry that precisely positions co-deposition of analytes and matrix so as to facilitate automated data acquisition.

[18] It is another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry which is optimal with respect to high sensitivity detection of analytes.

[19] It is another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry that retains liquid sample drop volumes of from less than 0.5 μL to greater than 10 μL , including liquid sample drop volumes greater than 3 μL known to be incompatible with prior art sample supports.

[20] It is another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry that retains liquid sample drops on the surface of the sample presentation device without the need for a physical reservoir or well.

[21] It is another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry that exhibits a minimum of nonspecific adsorption with respect to analytes.

[22] It is another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry that is suitable for the direct collection and subsequent analysis of fractions recovered from high performance liquid chromatographic (HPLC) separations.

[23] It is another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry that is suitable for the analysis of enzymatic digests prepared from protein spots excised from 1- and 2-dimensional electrophoresis gels.

[24] It is another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry that is suitable for the analysis of samples recovered from surface plasmon resonance (SPR) biosensors.

[25] It is yet another object of the present invention to provide a sample presentation device for matrix-assisted laser desorption/ionization mass spectrometry that is suitable for sample processing on standard multi-well plate processors and laboratory liquid handling robots.

[26] It is still yet another object of the present invention to provide for methods for using and creating the aforementioned devices. More specifically, it is an object of the present invention to use the electrowetting sample presentation devices of the present invention to identify the presence of analytes in a sample, and to analyze a plurality of samples, either on a sample presentation device or on a plurality of sample presentation devices.

[27] The above and other objects of the present invention are realized in specific embodiments of an electrowetting sample presentation device. The novel sample presentation device of the present invention is utilized as a sample collection device to enable receipt of liquid sample drop volumes of from less than 0.5 μL to greater than 10 μL . Volumes greater than about 3 μL are known to be incompatible with prior art sample supports, in that they are known to result in the heterogeneous deposition of analytes with a concomitant reduction in sensitivity of detection and ease of automated data acquisition. The novel sample presentation device of the

present invention is further utilized as a precise sample positioning device that confines the co-deposition of analytes and matrix to within a surface area measuring less than about one-half millimeter squared (0.5 mm^2). Confining the co-deposition of analytes and matrix is known to facilitate the homogeneous deposition of analytes with a concomitant increase in both ease of automated data acquisition and sensitivity of detection. As a result, the novel electrowetting sample presentation device of the present invention provides optimum utility with respect to liquid sample droplet receipt, sample positioning and confined co-deposition of analytes and matrix. In preferred embodiments, this combination of attributes affords an increase in sensitivity of detection of from about 10-fold to greater than 50-fold, as well as an increase in throughput of at least 10-fold, as compared to prior art sample supports.

[28] The sample presentation device of the present invention is comprised of a physical or virtual microwell which can receive a liquid sample drop; one or more intermediary electro-wettable sites at least one of which is contiguous to the microwell; and a terminal electro-wettable site which confines the deposition of analytes and matrix to within a predetermined area. Each of the electro-wettable sites modifies the surface of the sample presentation device between hydrophobic and hydrophilic states in response to an electrical potential applied between a liquid sample drop and the electro-wettable site so as to direct the positioning of the liquid sample drop. Furthermore, with respect to the path which originates at the microwell, the surface area of each succeeding intermediary electro-wettable site is equal to or less than that of the preceding electro-wettable site.

[29] The present invention further provides a sample presentation device having from 2 to 1536 individual sample presentation sites, wherein each sample presentation site is further comprised of a physical or virtual microwell which can receive a liquid sample drop; one or more intermediary electro-wettable sites at least one of which is contiguous to the microwell; and a terminal electro-wettable site which confines the deposition of analytes and matrix to within a predetermined area. Preferably, the sample presentation device of the present invention having many sample presentation sites is configured in a manner analogous to Life Science Industry's standard 96, 384 and 1536 multiwell plates, so as to be compatible with standardized multiwell plate processors and laboratory liquid handling robots.

[30] The physical or virtual microwell of the present invention provides a containment which holds a liquid drop when deposited therein. The containment can result either from a physical barrier or from a patterned zone exhibiting greater wettability (lower surface tension) than the surrounding area. Preferably, the surface of the microwell of the present invention is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes, or hydrophobic and adsorptive properties with respect to analytes. More preferably, the shape of the microwell of the present invention provides for some overlap between the edge of a liquid drop residing therein and one or more contiguous electro-wettable sites so as to facilitate the transfer of a liquid drop to adjacent electro-wettable sites.

[31] Once deposited into the microwell, a liquid sample drop containing dissolved analytes and matrix is allowed to evaporate until the volume of the drop is significantly reduced, and then transferred onto one or more adjacent electro-wettable sites by actuation of the appropriate electrodes. As the liquid drop continues to evaporate, it is repeatedly transferred along a path in which the surface area of each succeeding electro-wettable site is equal to or less than that of the preceding electro-wettable site. Lastly, the liquid sample drop (the volume of which has been significantly reduced owing to evaporation) arrives at the terminal electro-wettable site. As the liquid drop finally dries, analytes are deposited as a homogeneous thin film on the surface of the terminal electro-wettable site. As a result, the sample presentation device of the present invention enables the confined deposition of analytes with a concomitant increase in sensitivity of mass spectrometric detection. Furthermore, unlike prior art sample supports the confined deposition of analytes is to a great extent independent of the initial volume of the liquid sample drop residing within the microwell.

BRIEF DESCRIPTION OF THE FIGURES

[32] The above and other objects of the present invention will become apparent from consideration of the detailed description presented in connection with the accompanying drawings in which:

[33] FIGS. 1 through 6 depict representative arrangements of the microwell, intermediary electro-wettable sites and terminal electro-wettable site of the sample presentation

device of the present invention. FIG. 1 depicts a linear arrangement of electro-wettable sites which exploits wide electrodes. FIGS. 2 and 3 depict a linear arrangement of electro-wettable sites which exploits narrow electrodes. FIG. 4 depicts a non-linear arrangement of electro-wettable sites which exploits wide electrodes. FIGS. 5 and 6 depict an arrangement of electro-wettable sites wherein intermediary electro-wettable sites are contained within the area circumscribed by the electro-wettable site which is contiguous with the microwell.

[34] FIGS. 7*a* and 7*b* depict a cross-sectional view of a representative sample presentation device of the present invention which shows the arrangement of the non-conducting substrate, electrodes, dielectric film and hydrophobic thin film surface.

[35] FIGS. 8*a* and 8*b* depict a cross-sectional view of a representative sample presentation device of the present invention which shows the arrangement of the non-conducting substrate, electrodes, dielectric film and hydrophobic thin film surface, wherein the area of the first intermediary electrode overlaps the area of the virtual microwell to enable the efficient transfer of the liquid sample drop to the first intermediary electrode.

[36] FIGS. 9*a* and 9*b* depict a sample presentation device of the present invention having a plurality of sample presentation sites.

[37] FIG. 10 depicts a sample presentation device of the present invention configured in the Life Science Industry's standardized 96-well plate format.

[38] FIGS. 11*a* through 11*f* depict various electrode arrangements may be exploited for fabrication of the sample preparation device of the present invention.

[39] FIGS. 12*a* and 12*b* depict a cross-sectional view of a representative sample presentation device of the present invention which shows the arrangement of the non-conducting substrate, electrodes, dielectric film and hydrophobic thin film surface, wherein a thin metallic grounding electrode is patterned on the thin hydrophobic film surface.

[40] FIGS. 13*a* and 13*b* depict a cross-sectional view of a representative sample presentation device of the present invention which shows the arrangement of the non-conducting substrate, electrodes, dielectric film and hydrophobic thin film surface, wherein a thin metallic grounding electrode is patterned between the dielectric film and the hydrophobic thin film surface.

[41] FIGS. 14a through 14d and 15a through 15d illustrate the operation of representative sample preparation devices of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[42] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs.

[43] “Adsorption” refers to the process by which an analyte is retained on a surface as a consequence of interactions between the analyte and the surface.

[44] “Analytes” refers to one or more components of a sample which are desirably detected.

[45] “Laser desorption/ionization mass spectrometer” refers to a mass spectrometer that utilizes a laser as an ionization source to enable desorption of analytes.

[46] “Mass spectrometer” refers to an apparatus that measures a parameter which can be translated into mass-to-charge ratios (m/z) of ions formed when a sample is ionized into the gas phase.

[47] “Matrix” refers to a molecule that absorbs energy from an ionization source in a mass spectrometer to enhance desorption of analytes from the surface of a sample presentation device. Reagents frequently utilized as matrix for the detection of biological analytes include *trans*-3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA), α -cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHBA). Other suitable energy absorbing molecules are known to those skilled in this art.

[48] “Sample presentation device” refers to a device that is insertable into and removable from a mass spectrometer and comprises a substrate having a surface for presenting analytes for detection.

[49] “Sensitivity of detection” refers to the analytical limit at which an analyte can be routinely detected.

[50] “Surface” refers to the exterior or upper boundary of a body or a substrate that contacts the sample.

[51] “Surface tension” refers to a property of liquids in which a liquid drop deposited on a surface tends to contract the smallest possible contact area because of unequal molecular cohesive forces near the surface, measured by the force per unit of length.

[52] “Wettability” refers to the degree to which a solid surface is wetted by a liquid. With respect to water, high-energy surfaces are efficiently wetted and have relatively low contact angles, whereas low-energy surfaces are not wetted and have relatively high contact angles.

Overview

[53] The sample presentation device of the present invention is comprised of a physical or virtual microwell which can receive a liquid sample drop; one or more intermediary electro-wettable sites at least one of which is contiguous to the microwell; and a terminal electro-wettable site which confines the deposition of analytes and matrix to within a predetermined area. Each of the electro-wettable sites modifies the surface of the sample presentation device between hydrophobic and hydrophilic states in response to an electrical potential applied between a liquid sample drop and the electro-wettable site so as to direct the positioning of the liquid sample drop. Furthermore, with respect to the path which originates at the microwell, the surface area of each succeeding intermediary electro-wettable site is equal to or less than that of the preceding electro-wettable site.

[54] The present invention further provides a sample presentation device having from 2 to 1536 individual sample presentation sites, wherein each sample presentation site is further comprised of a physical or virtual microwell which can receive a liquid sample drop; one or more intermediary electro-wettable sites at least one of which is contiguous to the microwell; and a terminal electro-wettable site which confines the deposition of analytes and matrix to within a predetermined area. Preferably, the sample presentation device of the present invention having many sample presentation sites is configured in a manner analogous to Life Science Industry’s standard 96, 384 and 1536 multiwell plates, so as to be compatible with standardized multiwell plate processors and laboratory liquid handling robots.

[55] The physical or virtual microwell of the present invention provides a containment which holds a liquid drop when deposited therein. The containment can result either from a physical barrier or from a patterned zone exhibiting greater wettability (lower surface tension) than the surrounding area. Preferably, the surface of the microwell of the present invention is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes, or hydrophobic and adsorptive properties with respect to analytes. More preferably, the shape of the microwell of the present invention provides for some overlap between the edge of a liquid drop residing therein and one or more adjacent electro-wettable sites so as to facilitate the transfer of a liquid drop to adjacent electro-wettable sites.

[56] When the surface of the microwell is both hydrophobic and adsorptive, analytes can be selectively retained from low elutropic strength buffers and washed with either acidified water or low ionic strength buffers to remove detergents and salts known to interfere with mass spectrometric detection prior to application of matrix solution.

[57] The descriptions that follow are merely exemplary and do not limit the scope of the invention. With reference to FIG. 1, the sample presentation device of the present invention is comprised of a physical or virtual microwell 1 which can receive a liquid sample drop, one or more intermediary electro-wettable sites 2a and 2b, at least one of which is contiguous to the microwell, and a terminal electro-wettable site 3 which confines the deposition of analytes and matrix to within a predetermined area, wherein the surface of the microwell 1 is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes or hydrophobic and adsorptive properties with respect to analytes, wherein each of the electro-wettable sites 2a, 2b and 3 modifies the surface of the sample presentation device between hydrophobic and hydrophilic states in response to an electrical potential applied between a liquid sample drop and the electro-wettable site so as to direct the positioning of the liquid sample drop, and wherein the surface area of each succeeding intermediary electro-wettable site 2b is equal to or less than that of the preceding electro-wettable site 2a.

[58] With reference to FIGS. 2 and 3, the sample presentation device of the present invention is comprised of a physical or virtual microwell 1 which can receive a liquid sample drop, a plurality of intermediary electro-wettable sites 2, at least one of which is contiguous to the microwell, and a terminal electro-wettable site 3 which confines the deposition of analytes

and matrix to within a predetermined area, wherein the surface of the microwell 1 is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes, or hydrophobic and adsorptive properties with respect to analytes, wherein each of the electro-wettable sites 2 and 3 modifies the surface of the sample presentation device between hydrophobic and hydrophilic states in response to an electrical potential applied between a liquid sample drop and the electro-wettable site so as to direct the positioning of the liquid sample drop, and wherein the surface area of each succeeding intermediary electro-wettable site 2 is equal to or less than that of the preceding electro-wettable site.

[59] With reference to FIG. 4, the sample presentation device of the present invention is comprised of a physical or virtual microwell 1 which can receive a liquid sample drop, a plurality of intermediary electro-wettable sites 2a, 2b, 2c, 2d and 2e, at least one of which (2a) is contiguous to the microwell, and a terminal electro-wettable site 3 which confines the deposition of analytes and matrix to within a predetermined area, wherein the path which originates at the microwell 1 and terminates at the terminal electro-wettable site 3 is comprised of intermediary electro-wettable sites 2a, 2b, 2c, 2d and 2e which are not disposed along a straight line.

[60] With reference to FIGS. 5 and 6, the sample presentation device of the present invention is comprised of a physical or virtual microwell 1 which can receive a liquid sample drop, two or more intermediary electro-wettable sites 2a and 2b, at least one of which is contiguous to the microwell (2a), and a terminal electro-wettable site 3 which confines the deposition of analytes and matrix to within a predetermined area, wherein one or more of the intermediary electro-wettable sites (2b) are located within the area circumscribed by the electro-wettable site which is contiguous with the microwell 1.

Fabrication of Electrowetting Sample Presentation Devices

[61] The present invention can be realized through microfabrication technologies familiar to those skilled in the art. Preferably, each of the electro-wettable sites of the present invention is comprised of a substantially planar laminate having, respectively, a non-conducting substrate, an addressable electrode, a dielectric thin film and a hydrophobic thin film surface which contacts the liquid sample drop. The non-conducting substrate is preferably selected

from, but not limited to, one of silicon, glass, aluminum, steel and quartz. The addressable electrode is preferably selected from, but not limited to, one of chromium, copper, gold, indium tin oxide (ITO), platinum and silver. The dielectric thin film is preferably selected from, but not limited to, one of aluminum oxide, barium strontium titanate (BST), silicon nitride, Parylene C, Parylene N, polyimide, silicon dioxide, silicon nitride, spin-on glass (SOG) and titanium dioxide. Finally, the hydrophobic thin film surface is preferably selected from, but not limited to, one of polyethylene terephthalate (PET), ethylene trifluoroethylene (ETFE), polyvinylidene difluoride (PVDF), polyvinylfluoride (PVF), ethylenechloro trifluoroethylene (ECTFE), polytetrafluoroethylene (PTFE), polyurethane (PFA), Teflon AG and cyclized perfluoro polymer (CYTOP).

[62] The descriptions that follow are merely exemplary and do not limit the scope of the invention. With reference to FIGS. 7*a* and 7*b*, the sample presentation device of the present invention is preferably comprised of a non-conducting substrate 1, a physical or virtual microwell 2 having a chemically-modified surface 3 which exhibits either hydrophobic and non-adsorptive properties with respect to analytes or hydrophobic and adsorptive properties with respect to analytes, two or more intermediary electrodes 4*a* and 4*b*, at least one of which is contiguous to the microwell (4*a*), and a terminal electrode 5. The electrodes 4*a*, 4*b* and 5, as well as the surrounding area (with the exception of the area of the microwell) are covered with a dielectric film 6, which is further covered with a thin hydrophobic film 7.

[63] With reference to FIGS. 8*a* and 8*b*, the sample presentation device of the present invention is more preferably comprised of a non-conducting substrate 1, two or more intermediary electrodes 2*a* and 2*b*, and a terminal electrode 3. The electrodes 2*a*, 2*b* and 3, as well as the surrounding area are covered with a dielectric film 4, which is further covered with a thin hydrophobic film 5 except in the area corresponding to the microwell. The surface of the virtual microwell 6 is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes or hydrophobic and adsorptive properties with respect to analytes. The area of the first intermediary electrode 2*a* overlaps the area of the virtual microwell 6 to enable the efficient transfer of the liquid sample drop from the virtual microwell to the first intermediary electrode.

[64] With reference to FIG. 9*a*, the sample presentation device of the present invention is preferably comprised of a plurality of physical or virtual microwells 1 which can

receive liquid sample drops, each of which has associated with it two or more intermediary electro-wettable sites 2, at least one of which is contiguous to the microwell, and a terminal electro-wettable site 3 which confines the deposition of analytes and matrix to within a predetermined area, wherein the surface of the microwells 1 is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes or hydrophobic and adsorptive properties with respect to analytes, wherein each of the electro-wettable sites 2 and 3 modifies the surface of the sample presentation device between hydrophobic and hydrophilic states in response to an electrical potential applied between a liquid sample drop and the electro-wettable sites so as to direct the positioning of the liquid sample drops, and wherein the surface area of each succeeding intermediary electro-wettable site is equal to or less than that of the preceding electro-wettable site.

[65] With reference to FIG. 9b, the sample presentation device of the present invention is preferably comprised of a plurality of physical or virtual microwells 1 which can receive liquid sample drops, each of which has associated with it two or more intermediary electro-wettable sites 2, at least one of which is contiguous to the microwell, and a terminal electro-wettable site 3 which confines the deposition of analytes and matrix to within a predetermined area, wherein the surface of each of the microwells 5 is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes or hydrophobic and adsorptive properties with respect to analytes, and wherein the surface of each of the electrodes 2 and 3 is covered with a dielectric film which is, in turn, covered with a thin hydrophobic film 6.

[66] With reference to FIG. 10, the present invention further provides a sample presentation device having from 2 to 1536 individual sample presentation sites, wherein each sample presentation site is further comprised of a physical or virtual microwell which can receive a liquid sample drop; one or more intermediary electro-wettable sites at least one of which is contiguous to the microwell; and a terminal electro-wettable site which confines the deposition of analytes and matrix to within a predetermined area. Preferably, the sample presentation device of the present invention having many sample presentation sites is configured in a manner analogous to Life Science Industry's standard 96, 384 and 1536 multiwell plates, so as to be compatible with standardized multiwell plate processors and laboratory liquid handling robots.

[67] With reference to FIG. 11, a variety of electrode arrangements may be exploited for fabrication of the sample preparation device of the present invention. These arrangements include, but are not limited to, narrow electro-wetting electrodes (with respect to the diameter of the liquid drop) utilized without an upper cover plate (FIG. 11a), narrow electro-wetting electrodes utilized in conjunction with an upper cover plate (FIG. 11b), wide electro-wetting electrodes (with respect to the diameter of the liquid drop) utilized in conjunction with an upper cover plate (FIG. 11c), wide electro-wetting electrodes utilized in conjunction with an upper grounding electrode (FIG. 11d), wide electro-wetting electrodes utilized in conjunction with an upper grounding electrode having a hydrophobic thin coating to minimize nonspecific adsorption of analytes (FIG. 11e), and wide electro-wetting electrodes utilized in conjunction with an upper plane having a mirror-image set of electro-wetting electrodes (FIG. 11f). Alternatively, the electrowetting electrodes as well as the grounding electrode(s) may be fabricated into the same laminate device as described below.

[68] With reference to FIGS. 12a and 12b, the sample presentation device of the present invention is preferably comprised of a non-conducting substrate 1, two or more intermediary electrodes 2a and 2b, and a terminal electrode 3. The electrodes 2a, 2b and 3, as well as the surrounding area are covered with a dielectric film 4, which is further covered with a thin hydrophobic film 5 except in the area corresponding to the microwell (7). The thin metallic grounding electrode 6 is patterned on the surface of the hydrophobic film 5. Further, the surface of the virtual microwell 7 is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes or hydrophobic and adsorptive properties with respect to analytes. The area of the first intermediary electrode 2a overlaps the area of the virtual microwell to enable the efficient transfer of the liquid sample drop from the virtual microwell to the first intermediary electrode. The configuration illustrated in FIGS. 12a and 12b eliminates the need for either an upper cover plate or upper grounding electrode as illustrated in FIGS. 11b through 11f.

[69] With reference to FIGS. 13a and 13b, the sample presentation device of the present invention is more preferably comprised of a non-conducting substrate 1, two or more intermediary electrodes 2a and 2b, and a terminal electrode 3. The electrodes 2a, 2b and 3, as well as the surrounding area are covered with a dielectric film 4. The thin metallic grounding electrode 5 is patterned on the surface of the dielectric film 4, which is further covered with a

thin hydrophobic film 5 expect in the area corresponding to the microwell (7). Further, the surface of the virtual microwell 7 is chemically-modified so as to exhibit either hydrophobic and non-adsorptive properties with respect to analytes or hydrophobic and adsorptive properties with respect to analytes. The area of the first intermediary electrode 2a overlaps the area of the virtual microwell to enable the efficient transfer of the liquid sample drop from the virtual microwell to the first intermediary electrode. The configuration illustrated in FIGS. 13a and 13b eliminates the need for either an upper cover plate or upper grounding electrode as illustrated in FIGS. 11b through 11f. Furthermore, the thin metallic grounding electrode 5 may be patterned simultaneously with the virtual microwell (7), thereby simplifying the fabrication process.

Operation of Electrowetting Sample Presentation Devices

[70] Once deposited into the microwell, a liquid sample drop containing dissolved analytes and matrix is allowed to evaporate until the volume of the drop is significantly reduced, and then transferred onto one or more adjacent electro-wettable sites by actuation of the appropriate electrodes. As the liquid drop continues to evaporate, it is repeatedly transferred along a path in which the surface area of each succeeding electro-wettable site is equal to or less than that of the preceding electro-wettable site. Lastly, the liquid sample drop (the volume of which has been significantly reduced owing to evaporation) arrives at the terminal electro-wettable site. As the liquid drop finally dries, analytes are deposited as a homogeneous thin film on the surface of the terminal electro-wettable site. As a result, the sample presentation device of the present invention enables the confined deposition of analytes with a concomitant increase in sensitivity of mass spectrometric detection. Furthermore, unlike prior art sample supports the confined deposition of analytes is to a great extent independent of the initial volume of the liquid sample drop residing within the microwell.

[71] A liquid drop initially retained within the physical or virtual microwell of the sample presentation device wets the surface of one or more adjacent electro-wettable sites in response to an applied electrical potential. When the potential is applied, the contact angle associated with that portion of the liquid drop in contact with the electro-wettable site is instantaneously reduced due to the local change in surface tension which results from the trapping of ions at the interface between the liquid drop and the surface of the electro-wettable site. Electrowetting results from an increase in surface energy in the actuated electro-wettable

site as compared to that of the physical or virtual microwell, thereby facilitating the movement of the liquid drop from the microwell to one or more adjacent electro-wettable sites. Similarly, a liquid drop residing on one or more initially-actuated electro-wettable sites wets the surface of one or more adjacent electro-wettable sites in response to an applied electrical potential. If the electrical potential associated with the initially-actuated electro-wettable sites is discontinued while that associated with the newly-actuated electro-wettable sites is continued, movement of the liquid drop from one set of electro-wettable sites to an adjacent set of electro-wettable sites is facilitated.

[72] The descriptions that follow are merely exemplary and do not limit the scope of the invention. FIGS. 14 and 15 illustrate the operation of the sample preparation device of the present invention. The liquid sample drop initially resides in the physical or virtual microwell of the present invention (FIGS. 14a and 15a). The drop is allowed to dry with a concomitant reduction in volume and then transferred to the electro-wettable site which is contiguous with the microwell (FIGS. 14b and 15b). The drop is allowed to further dry with a further concomitant reduction in volume and then transferred to the adjacent electro-wettable site which is contiguous with the terminal electro-wettable site (FIGS. 14c and 15c). Finally, the drop is allowed to yet further dry and then transferred to the terminal electro-wettable site where the analytes and matrix are deposited as a thin film on the surface (FIGS. 14d and 15d).

Use of Electrowetting Sample Presentation Devices

[73] A significant increase in the sensitivity of detection results from the process described in FIGS. 14 and 15. In the absence of the electro-wettable sites, the average analyte surface concentration per unit area in the physical or virtual microwell is equal to the total analyte concentration divided by the surface area (assuming the deposited thin film of analyte has negligible thickness). In the presence of the electro-wettable sites, however, the deposition of analyte is confined to the terminal electro-wettable site wherein the average analyte surface concentration per unit area is equal to the total analyte concentration divided by the surface area of the terminal electro-wettable site (again assuming the deposited thin film of analyte has negligible thickness). Therefore, the presence of the electro-wettable sites and in particular the terminal electro-wettable site, affords an increase in average surface concentration of analyte which is equal to the ratio of the surface area of the physical or virtual microwell to the surface

area of the terminal electro-wettable site. Since the surface area of the terminal electrowettable site is always significantly smaller than the surface area of the physical or virtual microwell, confining analyte deposition to the surface area of the terminal electro-wettable site results in a significant increase in the average surface concentration of analyte presented to the mass spectrometer with a concomitant increase in sensitivity of detection.

[74] For example, the sample presentation device of the present invention with a microwell having a 3.0 mm diameter (about 7.069 mm² surface area) and a terminal electro-wettable site having a 0.25 mm² surface area, confines the deposition of analytes to a detection zone surface area of about 28-fold smaller than the surface area of the microwell, with an approximate 28-fold concomitant increase in average surface analyte concentration. Consequently, in principal the sample drop drying process described hereinabove would potentially afford an about 28-fold increase in sensitivity.

Analytes

[75] The sample presentation device of the present invention may be exploited to facilitate high sensitivity mass spectrometric detection of biological analytes selected from, but not limited to: biological macromolecules such as peptides, proteins, enzymes, enzymes substrates, enzyme substrate analogs, enzyme inhibitors, polynucleotides, oligonucleotides, nucleic acids, carbohydrates, oligosaccharides, polysaccharides, avidin, streptavidin, lectins, pepstatin, protease inhibitors, protein A, agglutinin, heparin, protein G, concanavalin; fragments of biological macromolecules set forth above, such as nucleic acid fragments, peptide fragments, and protein fragments; complexes of biological macromolecules set forth above, such as nucleic acid complexes, protein-DNA complexes, gene transcription complex, gene translation complex, membrane, liposomes, membrane receptors, receptor ligand complexes, signaling pathway complexes, enzyme-substrate, enzyme inhibitors, peptide complexes, protein complexes, carbohydrate complexes, and polysaccharide complexes; small biological molecules such as amino acids, nucleotides, nucleosides, sugars, steroids, lipids, metal ions, drugs, hormones, amides, amines, carboxylic acids, vitamins and coenzymes, alcohols, aldehydes, ketones, fatty acids, porphyrins, carotenoids, plant growth regulators, phosphate esters and nucleoside diphosphosugars, synthetic small molecules such as pharmaceutically or therapeutically effective agents, monomers, peptide analogs, steroid analogs, inhibitors, mutagens, carcinogens,

antimitotic drugs, antibiotics, ionophores, antimetabolites, amino acid analogs, antibacterial agents, transport inhibitors, surface-active agents (surfactants), amine-containing combinatorial libraries, dyes, toxins, biotin, biotinylated compounds, DNA, RNA, lysine, acetylglucosamine, procion red, glutathione, adenosine monophosphate, mitochondrial and chloroplast function inhibitors, electron donors, carriers and acceptors, synthetic substrates and analogs for proteases, substrates and analogs for phosphatases, substrates and analogs for esterases and lipases and protein modification reagents; and synthetic polymers, oligomers, and copolymers such as polyalkylenes, polyamides, poly(meth)acrylates, polysulfones, polystyrenes, polyethers, polyvinyl ethers, polyvinyl esters, polycarbonates, polyvinyl halides, polysiloxanes, and copolymers of any two or more of the above. Moreover, analytes that may be handled by the sample presentation devices of the present inventions may be non-biological.

[76] Analytes may be dissolved in aqueous buffers, organic solvents or mixtures thereof. Buffers are preferably selected from those prepared from volatile constituents including, but not limited to: ammonium acetate, ammonium bicarbonate, ammonium carbonate, ammonium citrate, triethylammonium acetate and triethylammonium carbonate, triethylammonium formate, trimethylammonium acetate, trimethylammonium carbonate and trimethylammonium formate. Aqueous samples containing high concentrations of non-volatile detergents (>0.1%) should be desalted prior to analysis as the presence of detergent may counteract and analyte-confining properties of the detection zone. Organic solvents are preferably selected from those known to be miscible in aqueous buffers and to promote the solubility of biological analytes including, but not limited to: acetic acid, acetone, acetonitrile, ethanol, *N,N*-dimethylformamide (DMF), *N,N*-dimethylsulfoxide (DMSO), formic acid, heptafluorobutyric acid, methanol, *N*-methylpyrrolidone (NMP), 2,2,2-trifluoroethanol and trifluoroacetic acid.

[77] Laser desorption time-of-flight mass spectrometry requires an energy absorbing molecule be applied to the surface of the sample presentation device to absorb energy and thereby effect the ionization of analytes. Energy absorbing molecules utilized in Matrix-Assisted Laser Desorption Ionization (MALDI) and Surface Enhanced Laser Desorption Ionization (SELDI) are frequently referred to as "matrix." Reagents frequently utilized for detection of biological analytes include *trans*-3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA), α -cyano-4-hydroxycinnamic acid (ACHA) and 2,5-dihydroxybenzoic acid (DHBA). Owing to

the limited solubility of the aforementioned matrix reagents in water, stock solutions of these reagents often contain 50% to 100% organic solvent.

[78] When utilized in conjunction with the sample presentation device of the present invention, stock solutions containing matrix reagents are added to aqueous samples prior to applying the sample to the surface of the sample presentation device. Alternatively, stock solutions containing matrix reagents may be applied to the surface of the sample presentation device after sample application and drying. In this instance, stock solutions containing a high percentage of organic solvent are preferably utilized to maximize dissolution of the analytes deposited on the surface of the microwell into the stock solution.

Applications of Electrowetting Sample Presentation Devices

[79] Applications of the sample presentation devices are described below. The descriptions that follow are merely exemplary and do not limit the scope of the invention. The sample presentation device of the present invention facilitates the mass spectrometric analysis of biological analytes recovered from fractionation schemes that exploit either column liquid chromatography or electrophoresis. In particular, utility results from the combination of the liquid-holding capacity of the device (which enables direct collection of chromatographic fractions, samples purified by electrophoresis, and samples recovered from biosensors without prior sample volume reduction) and the precise positioning of the sample and increased sensitivity of detection (which enables automated data acquisition). Furthermore, the availability of the sample presentation device in standard 96-well, 384-well and 1536-well formats enables sample collection and processing on multi-well plate processing devices and laboratory liquid handling robots. Consequently, the sample presentation device of the present invention may be exploited to enable high-throughput mass spectrometric platforms as are needed to support the emergence of proteomics and are currently unavailable.

[80] The liquid-holding capacity afforded by the sample presentation device of the present invention enables direct collection of fractions recovered from affinity chromatography, hydrophobic interaction chromatography, ion exchange chromatography, immobilized metal ion affinity chromatography and size exclusion chromatography, as well as fractions recovered from orthogonal separations involving sequential utilization of two or more of the chromatographic

approaches enumerated above. The volumes associated with fractions recovered from chromatographic separations involving biological analytes are usually in the range of from about 5 μ L to greater than about 100 μ L (unless recovered from nano-scale separations). Volumes greater than about 3 μ L are known to be incompatible with prior art mass spectrometer sample supports unless applied as small aliquots (e.g., 0.5 μ L) which must be allowed to dry prior to each application. Manual application of sample aliquots is both labor intensive and time consuming. Alternatively, protocols undertaken to reduce sample volume prior to sample application on prior art mass spectrometer sample presentation devices are labor intensive, time consuming, and often afford poor recoveries due to the loss of sample associated with the handling of small volumes.

[81] Contemporary protein quantification often involves enzymatic digestion of proteins purified either by column liquid chromatography or excised from 2-dimensional electrophoreses gels. Protein digests require desalting on reverse phase liquid chromatography (RPLC) columns prior to mass spectrometry. Unfortunately, automated desalting by high performance RPLC on both narrow-bore and micro-bore columns (2.1 and 1.0 mm ID, respectively) routinely affords sample volumes that are incompatible with prior art mass spectrometer devices used to store samples. The sample presentation devices of the current invention, on the other hand, are suitable for direct collection and subsequent analysis of protein digests desalted by high performance RPLC on both narrow-bore and micro-bore columns.

[82] The liquid-holding limitations known to be associated with prior art mass spectrometer sample presentation devices have prompted the development of various micro-column liquid chromatography approaches involving the use of small pipette tips packed with minute quantities of chromatographic media (e.g., ZipTips[®]). Micro-column approaches enable the desalting of protein digests with a concomitant reduction in sample volume reported to be sufficient to enable the sample to be applied directly to prior art mass spectrometer devices for retaining samples. However, the manual step-wise application of two or three aliquots is a far more common practice. The sample presentation devices of the present invention, on the other hand, are suitable for direct collection and subsequent analysis of protein digests desalted by micro-column RPLC.

[83] Surface plasmon resonance (SPR) biosensors exploit immobilized proteins to study protein-protein and other biological interactions. In principal, commercial biosensors and mass spectrometry are highly compatible in that the quantity of protein captured by interaction with an immobilized protein on a biosensor surface is a suitable quantity for mass spectrometric analysis. Although direct detection of analytes on the biosensor surface has been demonstrated, the elution of retained analytes from the biosensor system appears a much more attractive approach due to the fact that the biosensor surface is expensive and may be recycled many times. Unfortunately, a large volume of eluant is required to recover an analyte from a biosensor and the concentration of analyte in the eluant is far too low for optimum mass spectrometry. The sample presentation device of the present invention is suitable for direct collection of analytes recovered from biosensor systems and affords the increased sensitivity of detection required for routine mass spectrometric analysis. Additionally, the sample presentation device may be configured to a standard 96-well format so as to be compatible with sample collection devices already integrated into biosensor systems and can be exploited to enable automated sample collection for mass spectrometric analysis.

[84] It will be obvious to those having skill in the art that many changes may be made to the details of the above-described embodiments of this invention without departing from the underlying principles thereof. The scope of the present invention should, therefore, be determined only by the following claim.

Claim

1. A device for presenting liquid samples for mass spectrometry comprising:
 - a microwell adapted to receive a volume of liquid, wherein the liquid contains analytes,
 - at least one intermediate electro-wettable site contiguous with a portion of the microwell,
 - a terminal electro-wettable site which is adapted to confine the deposition of analytes in the liquid to a predetermined area, and
 - wherein the intermediate electro-wettable site is positioned between the microwell and the terminal electro-wettable site.

Abstract of the Disclosure

Figure 1

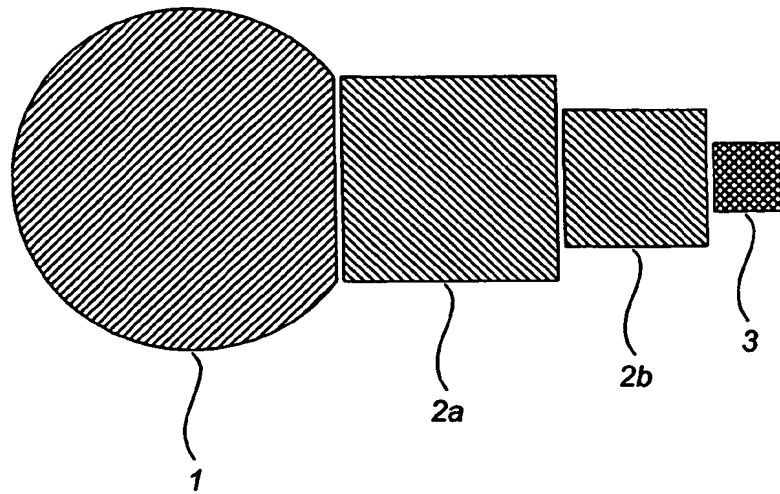


Figure 2

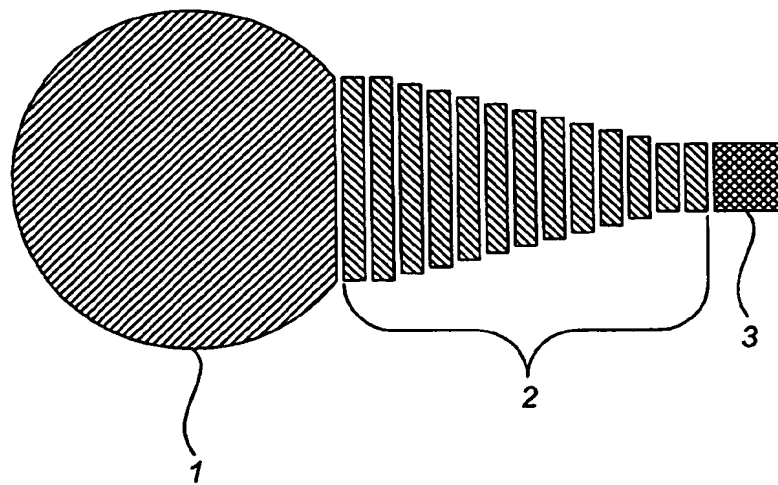


Figure 3

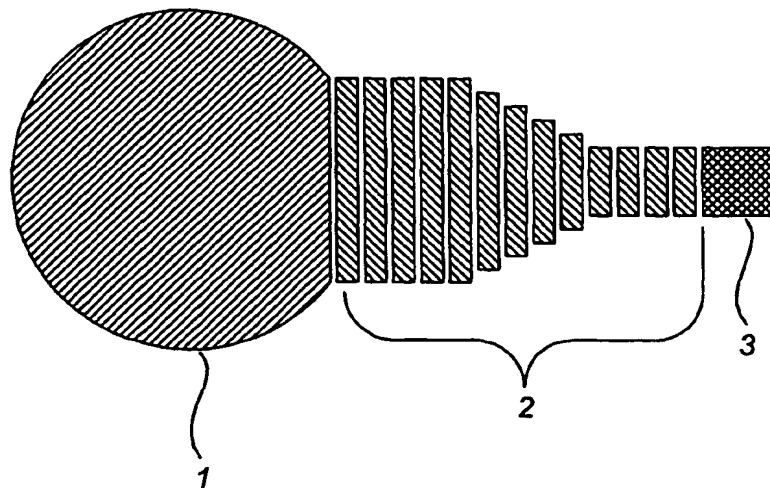


Figure 4

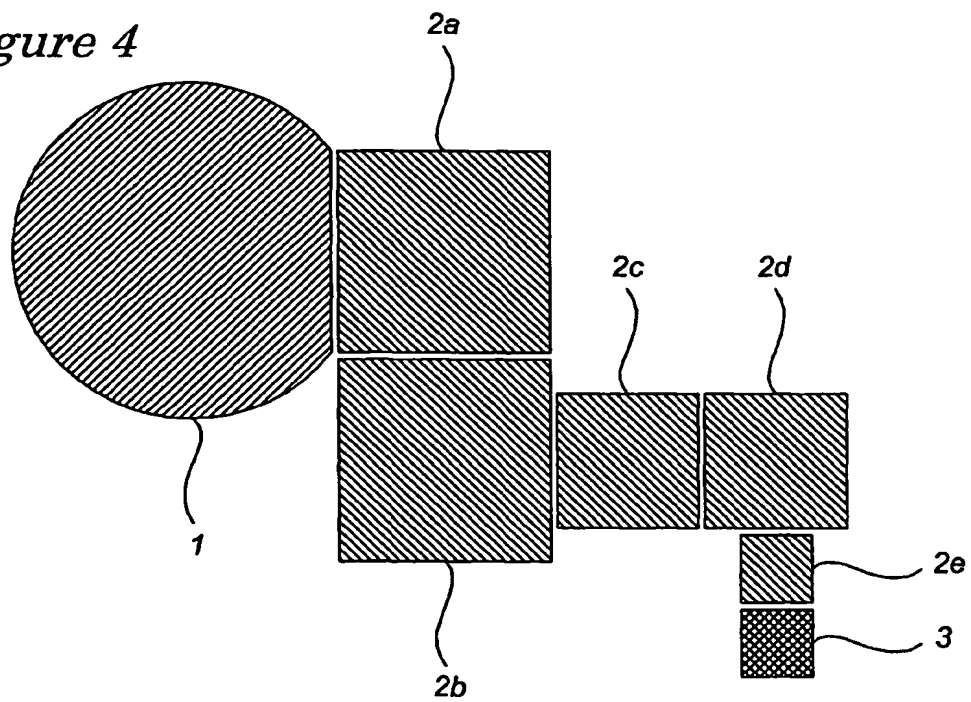


Figure 5

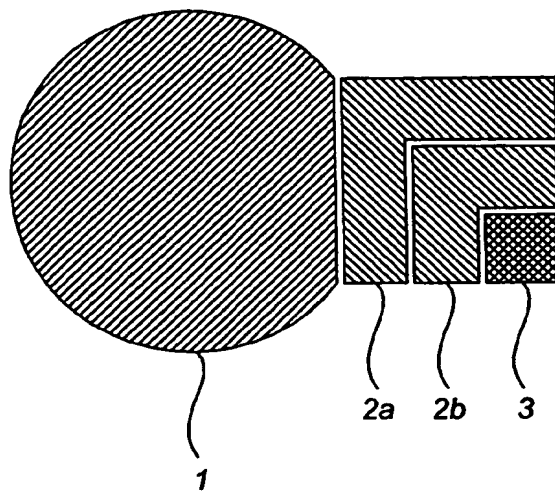


Figure 6

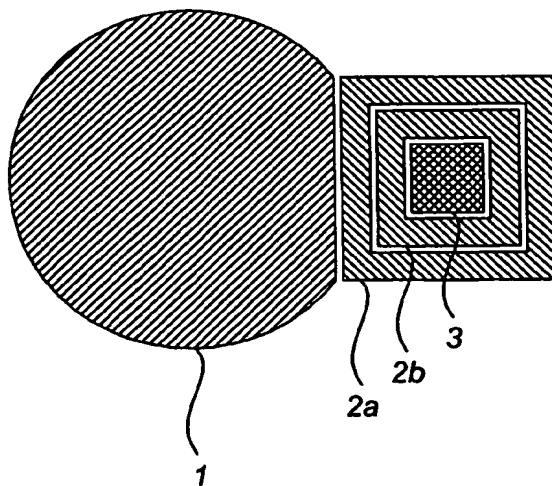


Fig. 7a

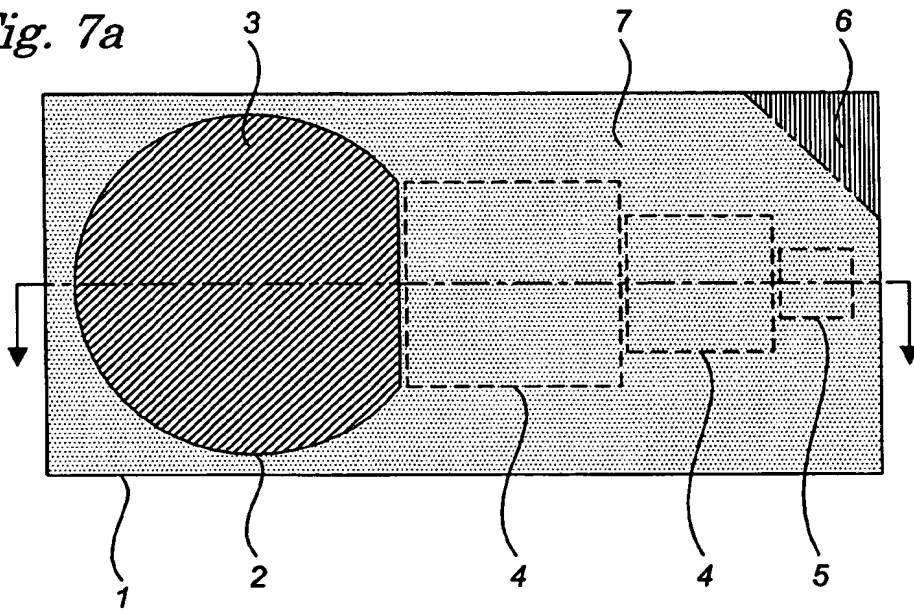


Fig. 7b

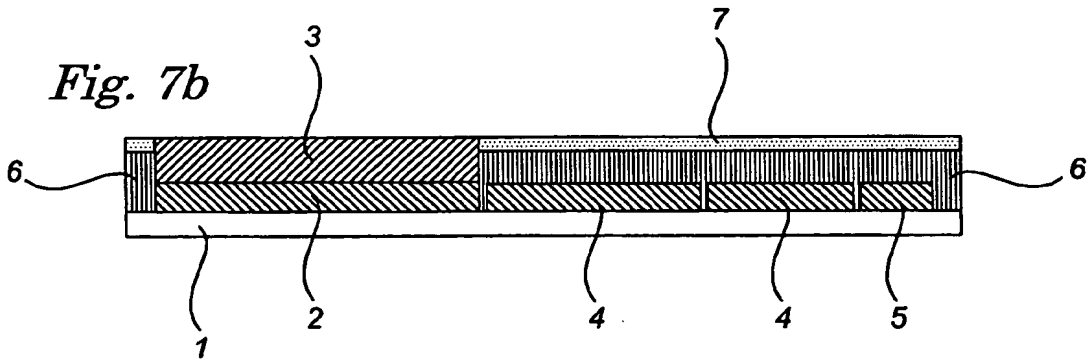


Fig. 8a

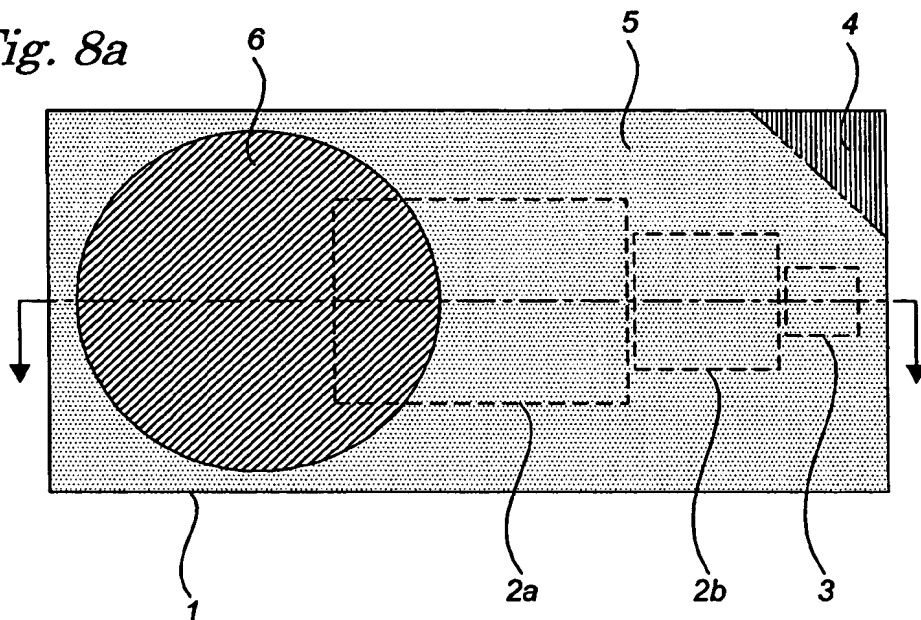


Fig. 8b

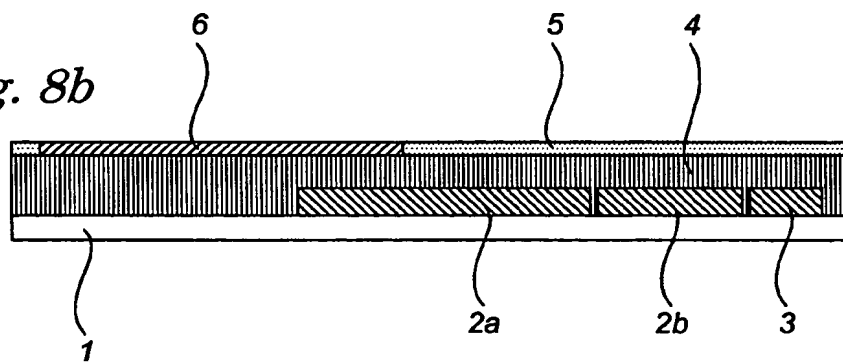


Fig. 9a

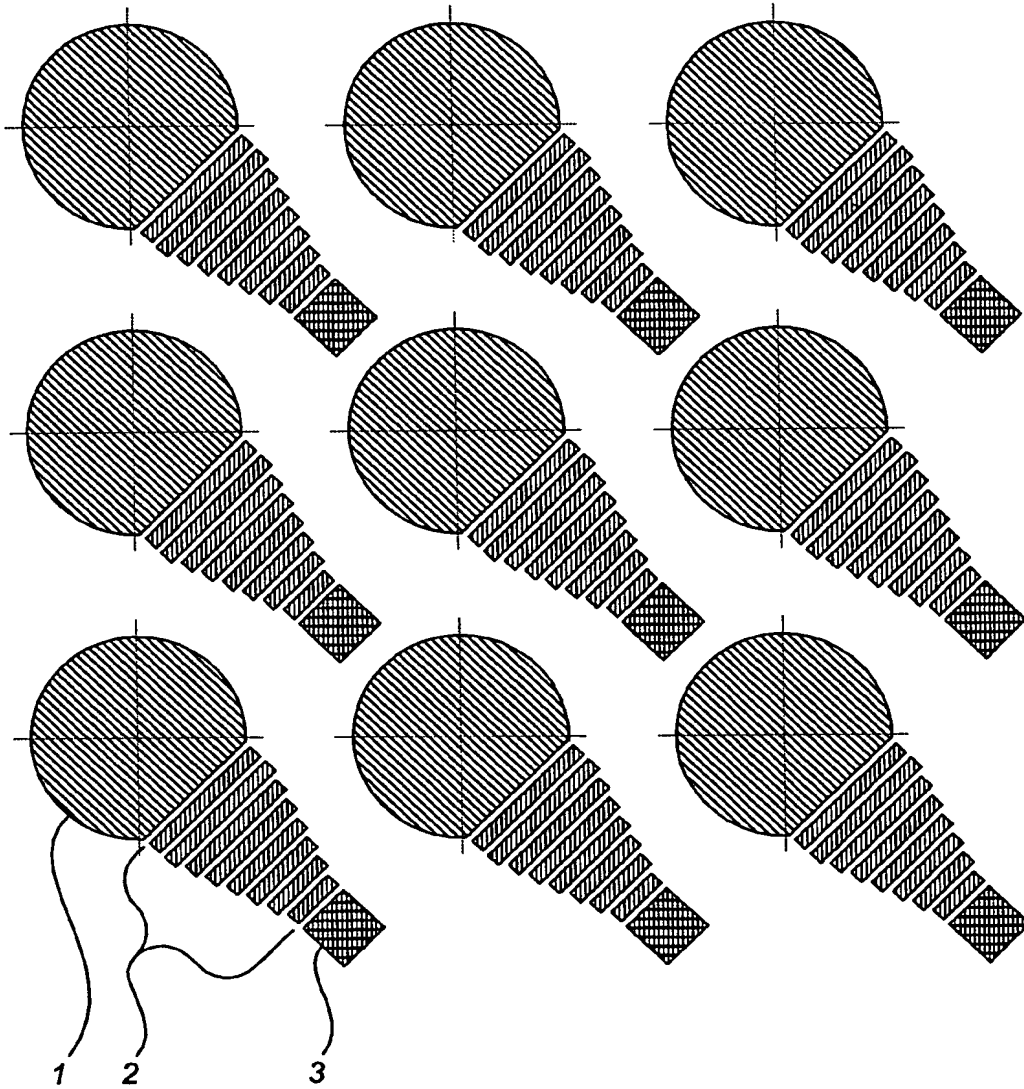


Fig. 9b

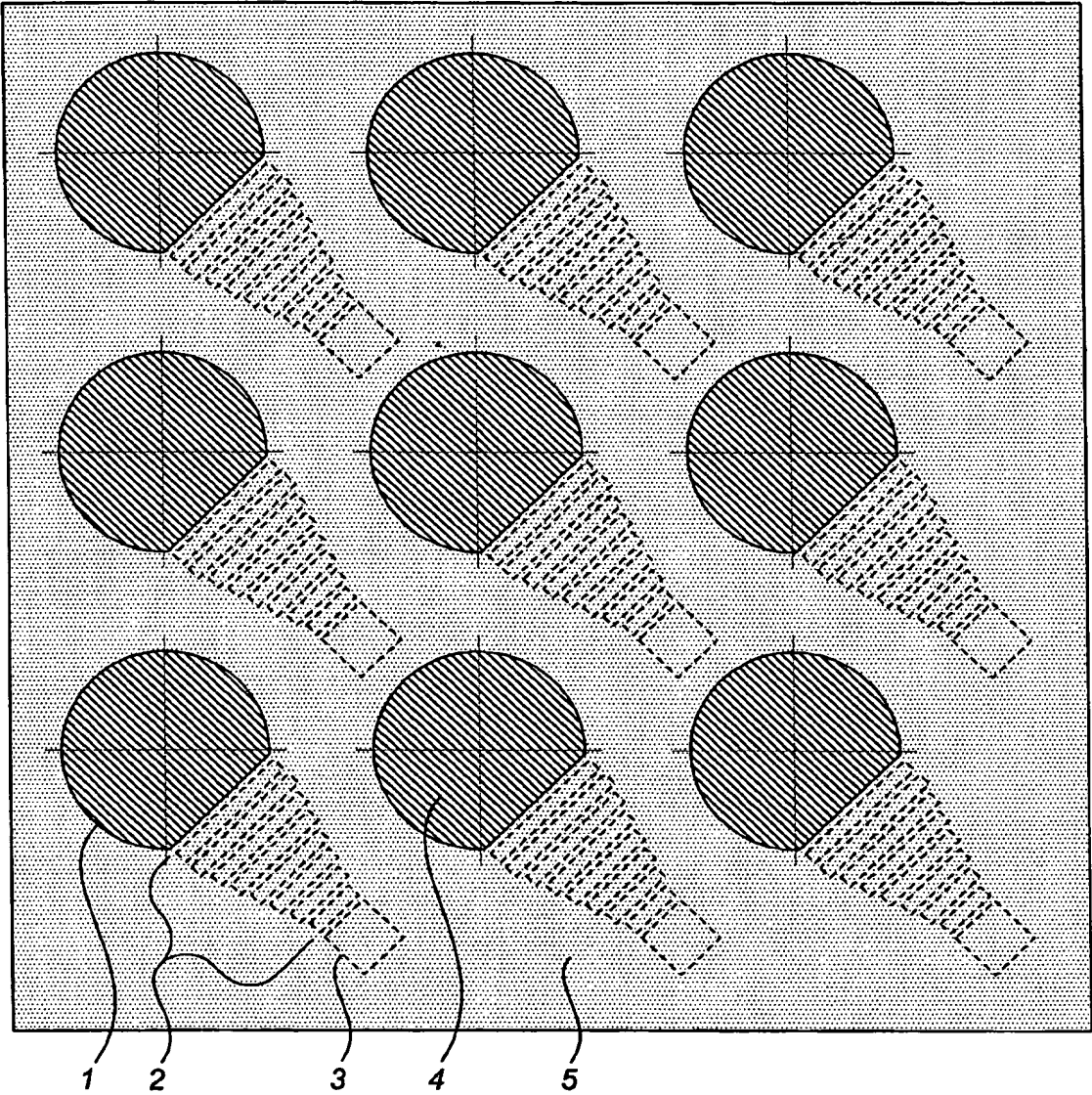


Figure 10

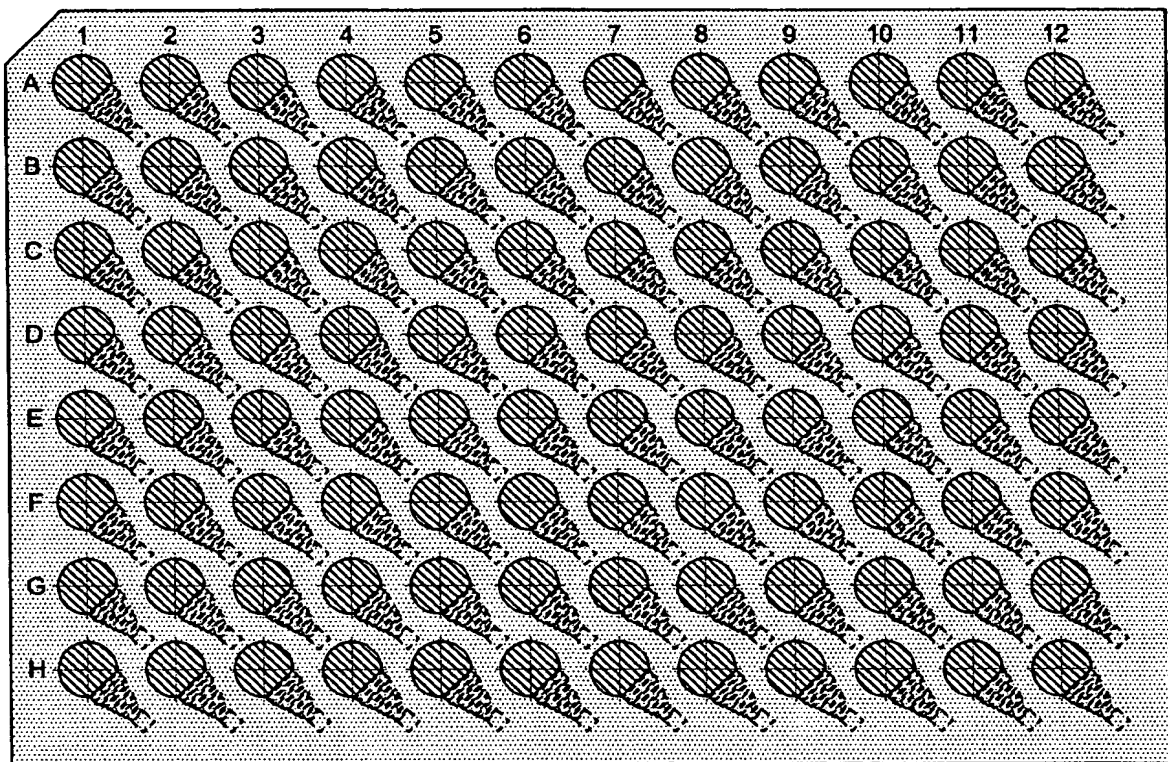


Fig. 11a

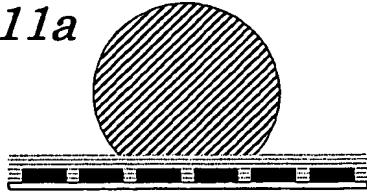


Fig. 11d



Fig. 11b

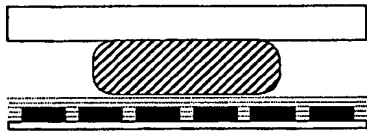


Fig. 11e

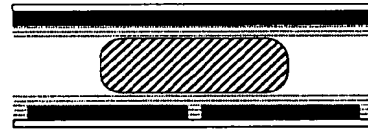


Fig. 11c

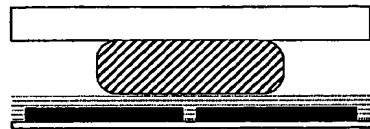


Fig. 11f

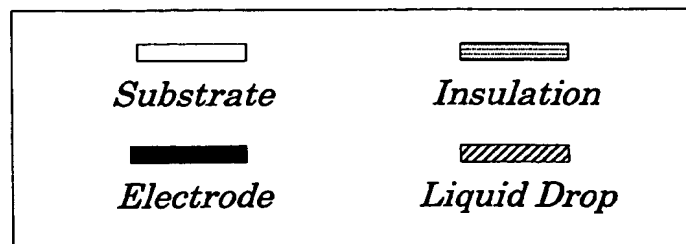
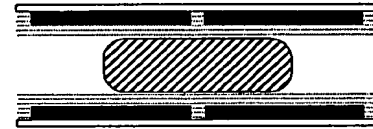


Fig. 12a

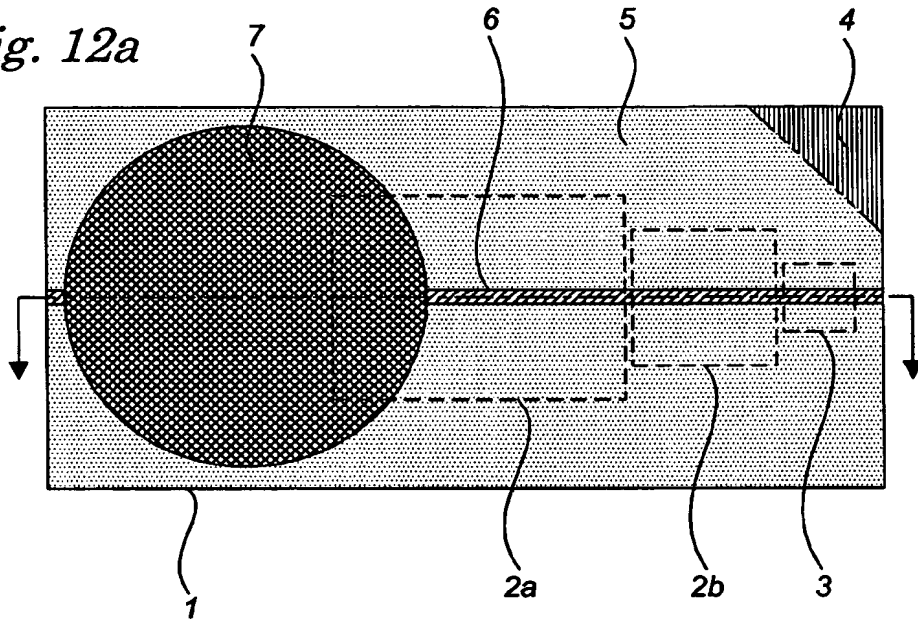


Fig. 12b

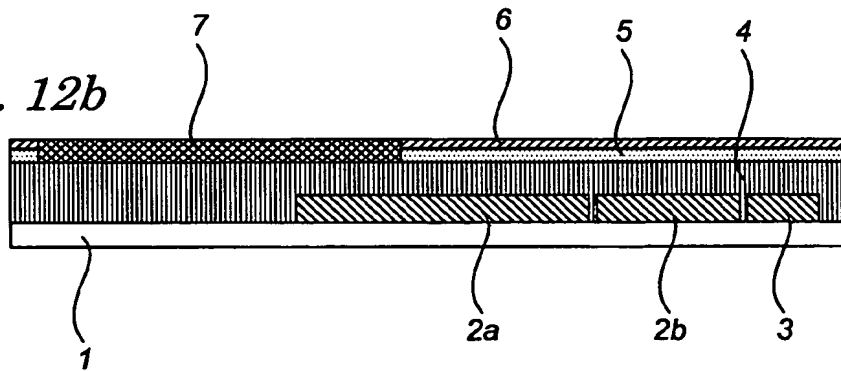


Fig. 13a

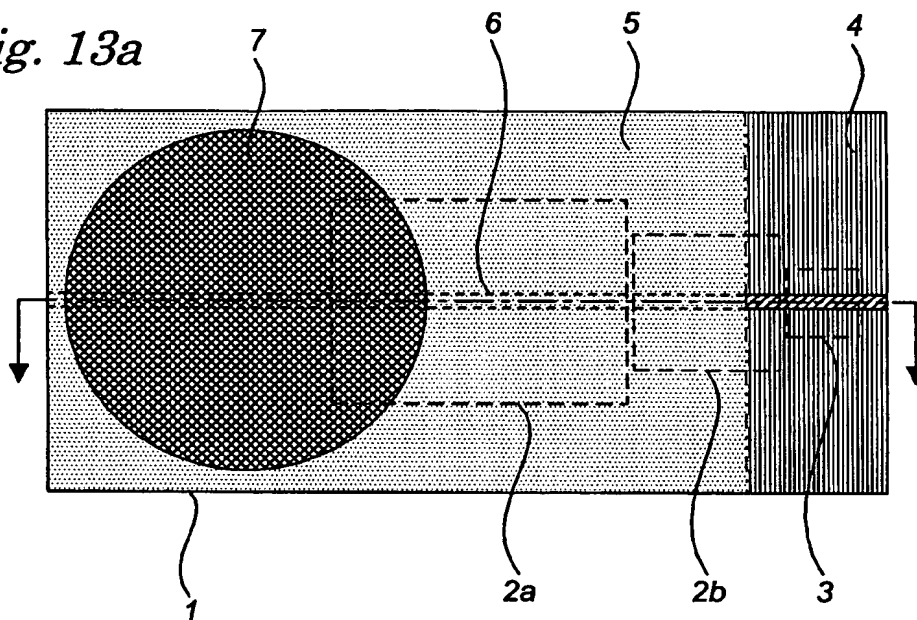


Fig. 13b

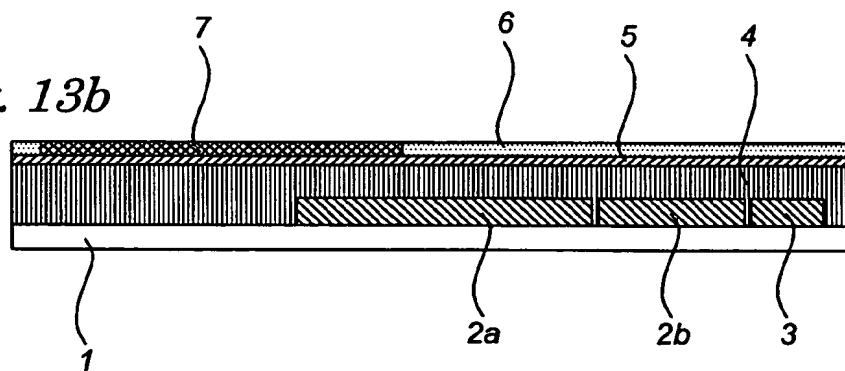


Fig. 14a

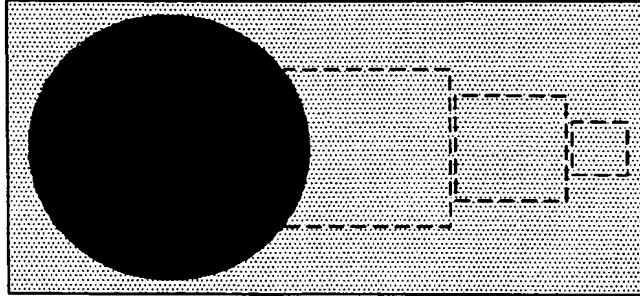


Fig. 14b

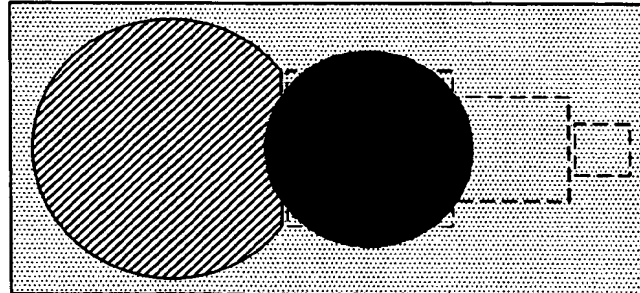


Fig. 14c

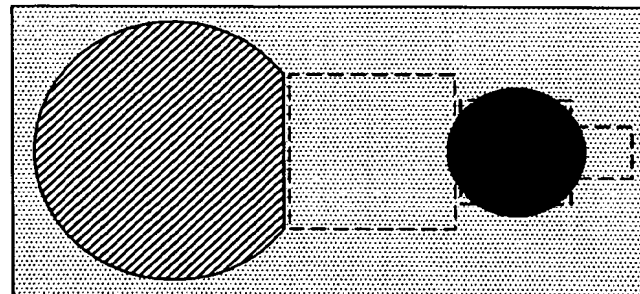


Fig. 14d

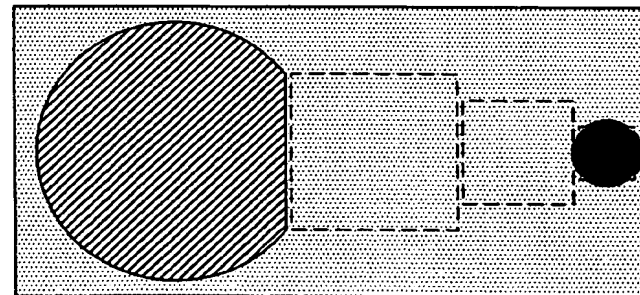


Fig. 15a

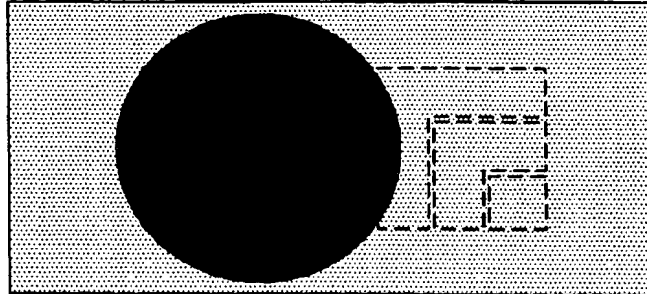


Fig. 15b

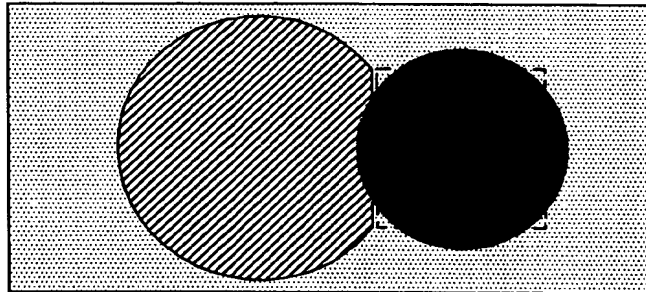


Fig. 15c

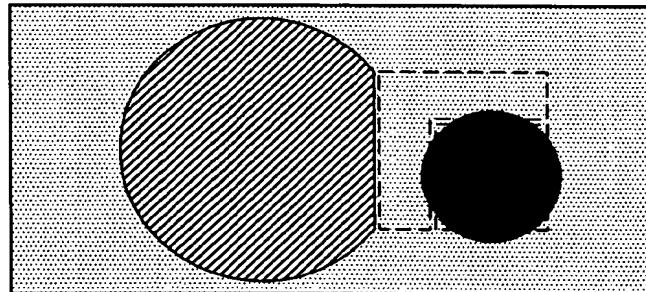
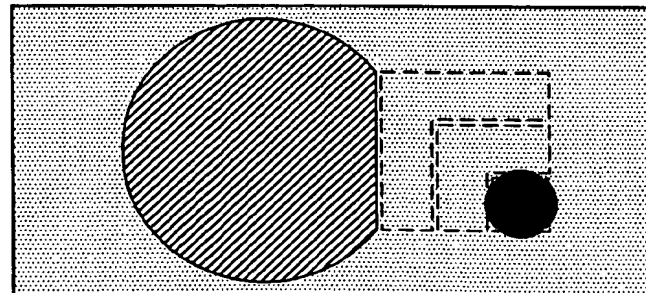


Fig. 15d



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